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A NEW SPECIES OF *PROCTOTRUPES* REARED FROM THE FERN WEEVIL (HYMENOPTERA, PROCTOTRUPIDAE).

By E. F. Riek.

[Read 25th May, 1955.]

Synopsis.

There is only one previous host record for an Australian representative of the family Proctotrupidae. A new species of *Proctotrupes*, which was reared from the fern weevil (*Syagrius fulvitarsus* Pascoe), is described in this paper.

Practically nothing is known of the biology of the Australian representatives of the family Proctotrupidae, so it is of interest to record at least the host of one of the species. The only other host record is for *Proctotrupes janthinae* (Dodd) which was bred from the larva of the fungus beetle, *Thallis janthina* (family Erotylidae).

Pemberton (1921) in a search for parasites of the fern weevil (*Syagrius fulvitarsis* Pascoe) in coastal New South Wales reared only the ichneumonoid *Ischiogonus syagrii* and the chalcidoid *Eupelmus* sp. The material on which the present study is based was not reared till 1929 and 1931 and nothing is known of the circumstances of its collection.

The weevil is known to attack the stems of many ferns including tree-ferns and bracken.

The parasite pupates in the skin of the host larva (note by L. Gallard).

PROCTOTRUPES SYAGRII, sp. nov.

Female. Shining black; legs including coxae all red, scape and pedicel pale and flagellum below pale, tegula pale.

Head, scutum and scutellum smooth, with fine pubescence; propleuron smooth, with a few fine, weak, irregular rugae at middle of anterior margin, otherwise glabrous laterally; mesopleuron all glabrous; parapsidal furrows distinct only anteriorly; propodeum rugoso-foveate over caudal half, anterior half with a strong median carina bordered by irregular foveae, laterad mostly glabrous; petiole very short; abdomen abruptly convex above from its base (in lateral view), ovipositor only about half as long as segment 2 of abdomen; forewing long, broad, slightly infuscated; pterostigma as wide as long, radial cell distinct, broadening at apical half; scape not quite as long as first funicle, pedicel quadrate, first funicle at least three times as long as wide, second subequal to first, succeeding segments decreasing, penultimate about one and a half times as long as wide, apical segment distinctly longer than scape.

Male. Legs all pale, scape and pedicel pale but flagellum all dark, tegula pale.

Similar to female but declivous portion of propodeum relatively larger and more coarsely and irregularly rugoso-foveate; antenna similar but funicle segments a little longer.

Types. Holotype ♀, allotype ♂ and 5 paratypes in the Entomology Branch Collection of the New South Wales Department of Agriculture. One paratype ♀ and one paratype ♂ in the C.S.I.R.O., Division of Entomology Museum.

Type Locality. Helensburgh, N.S.W. (3 ♀♀, 3 ♂♂) (July, 1931).

Locality records. New South Wales: Thirroul (23-i-1929, L. Gallard), 1 ♀; Coalcliff (23-i-1929, L. Gallard), 1 ♀; Coaldale (12-xi-1929), 1 ♂.

The glabrous propleuron without longitudinal rugae or sulci is most distinctive, as too are the completely pale legs. All other Australian species have some strong markings on the propleuron, and the legs are partly dark.

Reference.

Pemberton, C. E., 1921.—The Fern Weevil Parasite. Its life history and introduction to Hawaii. *The Hawaii. Plant Rec.*, 25: 196-201.

ESTIMATION OF PROTOZOAN POPULATIONS IN SOILS BY DIRECT MICROSCOPY.

By J. S. Bunt, Teaching Fellow in Microbiology, Microbiology Laboratory, University of Sydney, and Y. T. Tchan, Macleay Bacteriologist to the Society.

(Plate vi, B.)

[Read 27th July, 1955.]

Synopsis.

The present paper discusses briefly the need for a technique that will enable reliable estimations to be made of populations of protozoa in the soil. Such a technique is then described. Evidence in support of the method is included and a modification is explained, involving the use of the Gram stain, which enables more extensive information to be collected on the soil microflora.

Introduction.

It is a well-known fact that culture techniques for the estimation of microorganisms in soil give only a relative value because there is no universal medium which will allow the growth of all soil organisms. It is well known that this is also true of soil protozoa. The recent technique introduced by Singh (1946) is the least selective method but it is by no means able to give a total number of protozoa without selection, e.g. flagellates may not feed on bacteria (private communication by Dr. N. B. Singh). Direct microscopy is the logical method to overcome the selectivity of culture techniques. Most soil microorganisms can be estimated by direct microscopy—Conn (1918), Cholodny (1930), Winogradsky (1925), Thornton (1934), Jones-Mollison (1948), Strugger (1948), Rossi (1921), Blair (1945), Tchan (1953), Manniger and Vamos (1950), Vamos (1950). For protozoa there is no adequate technique. The use of the dark field microscope does not always allow a distinction to be made between protozoa and algae and motile bacteria. The presence of soil particles makes the use of dark field microscopy very difficult and, in some cases, impossible.

The staining techniques normally applied to the direct microscopy of soil are not adequate. Simple staining methods do not provide sufficient contrast for easy observation of soil protozoa at low magnifications. Further, the normally low numbers of protozoa in soil would render this type of technique quite inadequate. The technique described below provides a method suitable for the estimation of soil protozoa (Tchan and Bunt, 1954).

Technique.

Preparation of soil suspension.

Soil is suspended in a $^1/_{2000}$ solution of agar in the ratio 1 soil : 4 agar. If the bacterial population is also to be examined, the agar solution should be freed of bacteria by heating the solution with egg white in an autoclave and filtering on No. 1 paper. From this suspension a suitable series of tenfold dilutions is prepared with the same agar solution. It may be found necessary to use wide-mouthed pipettes for these manipulations to avoid blockages caused by large soil particles.

Preparation of slides.

From each dilution 0·1 c.c. is deposited on each of five clean slides. To prevent undue spread of suspension on the slides, it is recommended that the drops be placed in squares of a suitable dimension (e.g. 1·5 cm. × 1·5 cm.) drawn with a grease pencil on the glass. The slides are fixed in osmic acid or formalin vapour and then dried at 37°C. (about 45 minutes).

Staining technique.

(*a*) The soil slide is first flooded with erythrosin (1 part in 100 in 5% phenol) for 1–2 minutes. After gentle washing in running water it is counterstained with methyl green (0·1% aqueous solution) for a few seconds. Wash in water and dry in air. The preparation is then mounted in euparal or immersion oil for examination. The soil particles are stained green and the protozoa pink with purple nuclei. Flagella and cilia are pink and readily visible. Bacteria are purple. Fungal hyphae may be pink or purple. (Plate vi, B.)

(*b*) A variant of staining technique (*a*) allows the differentiation of Gram + and Gram – bacteria and protozoa in soil.

Since the Gram technique used has not been fully reported in the previous paper (Tchan, 1952), it is of interest to give some details here. The principle of using iodine-alcohol to avoid excessive differentiation is not new. The difficulty with soil preparations resides in the strong affinity of some soils for crystal violet, a prolonged washing with alcohol being required to remove the dye from soil colloids. It is necessary to use a well-defined iodine-alcohol solution which should enable a very efficient decolorization of soil colloids but not of Gram-positive microorganisms.

After several trials it was found that the formula used is most suitable. Some slides kept in the iodine-alcohol solution overnight still showed some Gram-positive organisms perfectly black. Usually the differentiation requires about 1–5 minutes to remove completely the crystal violet from the soil particles. However, it has been found that some soils require 10 minutes.

Some reputed Gram-variable bacteria, e.g. *Corynb. diphtheriae*, were found to remain Gram-positive after washing with iodine-alcohol. When the iodine concentration is too low or too high, the Gram-positive organisms may be decolorized or the Gram-negative organisms may remain black.

When applying the stain to agar films in the Jones and Mollison technique (1948), it is necessary to ensure that the agar film is perfectly dry. It is advisable to pass the slide over a flame for a few seconds and allow to cool before the staining process.

Technique (Tchan, 1952).

(1) Crystal violet 10 g., ammonium oxalate 4 g., 95% alcohol 100 c.c., water 400 c.c.
(2) Iodine 1 g., KI 2 g., 95% alcohol 25 c.c., water 100 c.c.
(3) Iodine solution as above 5 c.c. + alcohol 95% 95 c.c.
(4) Erythrosin 1 g., phenol 5 g., water 100 c.c.

The slide preparation is flooded by (1) for one minute, wash with tap water, stain one minute with iodine solution (2). Wash with iodine solution (3) until no more violet colour can be removed. Wash with water. Counterstain with erythrosin (4). Wash in water. Counterstain with methyl-green as in method (*a*) above.

The Gram + bacteria are blue or black, and Gram – bacteria purple (due to the combined colour of erythrosin and methyl-green). The other microorganisms are stained as in the method (*a*).

Counting technique.

Examine the slides with a 10× objective for large protozoa and 40× for small protozoa. If necessary, the 65× objective can be used for checking purposes. Count the number of slides containing protozoa (record presence or absence only) until the last dilution gives a negative result in all five slides. The number of protozoa is calculated from McGrady's probability tables (1948, see Calmette et al.).

Recovery tests.

With a culture of *Colpoda* sp. the number per unit volume of suspension was estimated first by fixing drops of the suspension on a slide in formalin vapour and counting the total number of protozoa before drying. After counting, the slides were allowed to dry and then stained with technique (*a*). The total numbers of protozoa in the drops were recounted. The results are summarized in Table 1.

The results show that very few cells were washed away during the staining process.

TABLE 1.
Recovery of Protozoa in Drops of Culture Stained by Technique (a).

Numbers of *Colpoda* sp. Before Drying.	Numbers of *Colpoda* sp. After Drying and Staining.
Drop 1 .. 152	Drop 1 152
,, 2 .. 100	,, 2 90
,, 3 .. 124	,, 3 132

Three cultures of protozoa were mixed in water. Their respective numbers were estimated by hæmacytometer counts. A known volume of the suspension was added to a known weight of soil. The recovery from the soil suspension was made according to the technique. The results are summarized in Table 2.

TABLE 2
Recovery of Protozoa Introduced into a Soil.

	Calculated Number.			Recovery.			
Experiment No.	1	2	Mean.	1	2	3	Mean.
Ciliates	84	87	86	125	45	85	85
Amoebae ..	27	29	28	35	20	35	30
Rhizopods ..	6	17	12	15	10	5	10
Total ..	117	133	126	175	75	125	125

These results show that the presence of soil particles does not interfere with the counting technique.

TABLE 3.
Counts of Protozoa in Two Soil Types.

	Lucerne Broth.	Mannitol Soil Extract Agar.	Direct Microscopy.
(*a*) University Garden Loam.			
Ciliates	275	6250	1750
Flagellates	350	6250	4000
Amoebae	0	2380	1750
Rhizopods	0	0	350
Spores	0	0	5500
Total	625	14880	13350
(*b*) Macquarie Island Peat Soil.			
Ciliates	5	63	850
Flagellates	0	0	250
Amoebae	0	0	250
Rhizopods	0	0	120
Spores	0	0	4000
Total	5	63	5470